1966

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James Dennis Slavin Jr.
Yale University

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UPTAKE AND DISTRIBUTION OF LSD-25
IN THE BRAIN OF THE
GUINEA PIG

James Dennis Slavin, Jr.

Thesis submitted in partial fulfillment
of the requirements for the degree
of Doctor of Medicine

Department of Psychiatry
Yale University School of Medicine
1966

Acknowledgements

The author wishes to express his gratitude to Dr. Daniel X. Freedman for his invaluable assistance and continued interest in this study.

The theoretical and technical assistance of Mr. Stephen Pachl and Mr. Karl Rozitis were invaluable in the undertaking and completion of the project.

TABLE OF CONTENTS

Introduction and Review

LSD was first synthesized and studied pharmacologically in 1938, by Stoll and Hofmann, who were at that time primarily attracted by its close structural resemblance to ergonovine, a naturally occurring ergot alkaloid. (44) Initial investigation, however, indicated that LSD was less potent and more toxic than ergonovine and thus not likely to be of clinical value as an oxytocic agent. In 1943, Hofmann accidentally ingested or, more probably, inhaled a small amount of LSD and thus discovered its remarkable and unsuspected psychogenic properties.

D-lysergic acid diethylamide, or d-LSD-25, is but one of four stereoisomeric alkaloids that can be synthesized from lysergic acid, the other stereoisomers being L-lysergic acid diethylamide and D- and L-isolysergic acid diethylamide; LSD, however, alone possesses the psychotomimetic properties that have been of such great interest to investigators for the last two decades. (29) LSD can be found naturally as a component of the ergot alkaloids produced when the fungus Claviceps purpurea attacks various grasses, principally rye. It is also found in the seed coats of various flowering plants, most notably the morning glory (Pearly Gates, Ipomoea vulvo, and Rivea Corymbosa). (29)

Pharmacology

Several investigators have studied the fate of LSD-25 administered to experimental animals. Boyd (11) estimates that only 10-20% of intraperitoneally injected LSD, labeled with C^{14} reached the circulation of the rat; he noted that organ levels of radioactivity were three times higher after intravenous administration, but that the same distribution pattern was followed. Rothlin (39) reported that LSD was not bound to plasma proteins in rat blood, but Boyd (11) found that a large percentage of LSD in plasma was so bound - 70% at 5 minutes, 40% at 3 hours. Axelrod (8), using a fluorometric assay, noted that 90 minutes after the intravenous injection of LSD in cats, the highest concentration was found in the bile, with decreasing concentrations present in plasma, lung, liver, brain, CSF and fat.

A survey of the literature indicates that surprisingly little effort has been made to determine the distribution of LSD in the brain of experimental animals, even though it seems obvious, as Reivich and Snyder (43) note, that "some of the characteristic clinical effects of LSD may be related to its regional localization in the brain." These authors used the fluorometric assay of Axelrod (7) to investigate this problem in the squirrel monkey; they found that LSD was in relatively low concentration in the cerebral cortex while the hypothalamus, visual and auditory reflex centers contained three times the cortical levels. These authors felt that the observed difference in the

distribution of LSD in the brain was not due to differences in blood flow or simply the result of regional differences in lipid solubility.

Unpublished data from the N.I.H. (13) indicates wide differences in the regional concentration of LSD in brains. If the frontal grey matter of the cerebral cortex is considered as having one "unit" of LSD per gram, then other regions may be compared to this norm, as follows. The lowest concentration was found in the dentate nucleus of the cerebellum (.77) and the highest concentrations were noted in the iris (18.3), anterior pituitary gland (10.5) and the pineal gland (6.85). Other significant areas included the hypothalamus (2.95), diencephalon (2.3) and the brainstem (1.1).

Freedman and Coquet (18) have recently studied the regional and subcellular distribution of LSD in rabbit brains. They found that the drug was taken up most readily by the hippocampus, where drug levels were not significantly modified by increasing the dose of LSD - an observation which was not noted in the caudal brain stem and diencephalon. The authors also found that 5 minutes after the intravenous administration of 500 micrograms of LSD per kilogram of rabbit, 65% of the LSD found in the brain was in the particulate fraction (largely in the microsomal component) and only 35% was in the supernatant.

Metabolism

The metabolism of LSD both in vitro and in vivo has been extensively investigated. Using tissue slices, Axelrod et al. (8) observed that only the liver was capable of metabolizing LSD. Stoll et al. (45) found that 70% of the total dose (in terms of LSD-C^{14}) was excreted into the bile within 6 hours of administration and that the bile contained less than one percent unchanged LSD and three (or possibly four) distinct metabolic break down products. Boyd (11) showed that at 36 hours, 8% of the C^{14} from labelled LSD appeared in the urine, 4% as $CO_2{}^{14}$ in expired air, and over 80% in the feces.

LSD alters the general body biochemistry in several directions. Siva Sankar (40) found that LSD increases the level of inorganic phosphorus in the blood and decreases urinary phosphorus output, an alteration that has been described but not confirmed as occurring in schizophrenics. Hollister (30) noted mobilization of free fatty acids, possibly due to norepinephrine and a reduction in the total eosinophil count. Abood (1) described an uncoupling of oxidative phosphorylation in carbohydrate metabolism. Sankar (40) also found that, in rats, LSD decreased food intake, decreased urea excretion, increased ammonia excretion and decreased the excretion of creatinine, keto-acids, sodium, potassium, and total amines.

Rothlin (39) has summarized the pharmacologic properties of LSD. Peripherally, he notes constriction of the uterus, both in vivo and in vitro,

as well as the constriction of blood vessels and bronchial musculature; he also notes that LSD is an inhibitor of the effect of 5-HT (serotonin) on different structures, both *in vivo* and *in vitro*. Autonomic effects are numerous and include mydriasis, elevation of body temperature, hyperglycemia, pilomotor erection, hypotension and respiratory depression.

Electrical Activity of the Brain

In the rat, Freedman *et al.* (21) have shown that no alteration in hippocampal activity can be detected with bipolar EEG recordings; the cortex, however, shows absence of spindling and spiking and an alert period. They also found that the EEG changes developed tolerance, although the time required was slightly longer than required for some of the behavioral changes. This was the first observed instance of tolerance to the EEG effects of drugs.

Evarts (15) has reviewed the neurophysiologic effects of LSD in several species. In rabbits, small doses of LSD (1-5 mcg. per kilogram) decreased the amplitude of the EEG and increased the frequency; larger doses (20-60 mcg. per kilogram) resulted in the reappearance of slow waves; similar changes were observed in cats. Aley (4) has described EEG changes in the limbic system of cats that lasted for several weeks.

Hoffer (29) has reviewed the changes in the human EEG produced by LSD. Alterations have been slight and included suppression of alpha activity and an increase in frequency in most subjects. Monroe *et al.* (36) employed

depth electrodes in humans rather than the usual scalp leads and showed that LSD caused the appearance of a "schizophrenic-like" pattern in the hippocampus, amygdaloid and septal areas of the brain even when the surface EEG was apparently normal.

Effect of LSD-25 in Humans

The effect of LSD-25 on normal human volunteers has been reviewed at length by Hoffer (29). He states that different subjects varied widely in their reactions to a given dose, ranging from those who noticed no effects to those who described fantastic experiences. There are many variables which affect and determine the nature of the response in any given individual. Concerning the most obvious of these variables - *i.e.*, the amount of LSD administered - there is some confusion in the literature. Rawnsley and Anderson (5) proposed that beyond a certain minimum dose, there was no clear-cut relationship, either quantitative or qualitative, between the clinical effect and the amount of LSD taken. Abramson (2), as well as Isbell (31), however, found that increasing the dose of LSD from 0-225 micrograms increased both the number and subjective severity of the symptoms. Concerning the variable of dosage, Klee *et al.* (32) reached the following conclusions:

1. The severity of the psychophysiological effects of LSD in a given subject is proportional to the dose administered.

2. Certain types of reactions to LSD, such as paranoid ideation, occur only occasionally, and appear to be less a function of dosage than of personal predisposition.
3. Even with respect to the fundamental effects of LSD, there is considerable variability among subjects in the severity of their reaction to a particular dose.

Other major variables which affect the reaction to LSD are personality, education, physical type, reason for taking the drug, mood, psychiatric history, the physical environment, and the number of people present.

Certainly the most spectacular subjective property of LSD is its hallucinogenic activity, first noted by Hofmann in 1943, and since investigated and described by numerous workers. (9,12,22,31,32,33) It was originally held that these phenomena resulted from central stimulation of undefined cerebral areas, but it was later noted that LSD was highly concentrated in the retina, optic nerve, and optic chiasm of experimental animals; thus it was proposed that the hallucinations were related to peripheral, not central, stimulation of the visual pathway - thus, Hoffer states that "blind people do not have vivid visual perceptual changes (after LSD)." Krill (33), however, reported on his work with 24 totally blind subjects and concluded that a normal retina was not necessary for the occurrence of LSD induced visual experiences; he did find that such visual phenomena occurred only in blind

subjects who previously had had sight and did not occur in subjects blind from birth or early childhood. Krill also noted that auditory, tactile, olfactory, and gustatory hallucinations occurred much more frequently in his blind subjects than is usually reported in people with normal vision.

LSD commonly interferes with intellectual processes in normal subjects, the most commonly observed derangements being shortened concentration span, impaired judgement, decreased learning ability, and poor performance of problem solving. Changes in mood may include euphoria, complete lack of emotion, depression, fear and, rarely, impulsive and bizarre behavior.

Cohen (12) has summarized the side effects and complications of controlled LSD ingestion after analyzing the results of 44 investigators who had collectively administered over 25,000 doses to more than 5,000 subjects. He found only eight instances of psychotic reactions lasting more than 48 hours, and only one occurred in a normal experimental subject, and it subsided within a few days. Suicides were very rare, and all occurred in psychiatric patients, rather than in normal volunteers. No instances of serious prolonged side effects were found in this study or in the literature. Adverse responses tended to occur at the higher dose levels, _i.e._, above 75 micrograms, but this was by no means invariable. Unpleasant reactions to LSD were encountered more frequently in subjects with excessive initial apprehension, rigid defense structures and significant subsurface guilt and conflict. No instance of

addiction was reported - furthermore, the author felt that physiological addiction was unlikely because of the rapid development of tolerance.

Frosch *et al.* (22) have recently reported on the untoward reactions to LSD suffered by those ingesting the drug in the community under uncontrolled and unsupervised circumstances. Twelve patients were extensively studied; it was found that all had had some degree of personality disorder before taking the drug and that five were definitely psychotic before their LSD experience. Adverse reactions in these patients included acute panic reactions, recurrence of LSD symptoms in a period of abstinence after multiple ingestion, and prolonged psychosis. The authors commented that the prognosis in the first group was good, but that no long-range follow-up results were available.

Mechanism of Action

Numerous theories have been proposed to explain the central nervous system, or psychotomimetic, mechanism of action of LSD; however, their very multiplicity and diversity is indicative of the uncertainty that is still dominant in this field. The answers and explanations have been sought in several areas of investigation, and a representative, but not comprehensive, review is presented.

Cellular Mechanisms

Geiger (23) studied the effect of the addition of minute quantities of LSD (0.001 mg./ml.) to the nutrient media of mammalian brain cell cultures

and observed a direct effect on the exposed cells. He found that within fifteen to twenty minutes the amount of Nissl substance, generally considered an indicator of cellular health, markedly decreased and that other granular elements in the cell became dispersed throughout the surrounding cytoplasm. The body of the neuron itself showed some evidence of contraction, and axonal and dendritic processes retracted. The nuclei became denser, while the nucleoli enlarged or became irregular in shape.

It was also noted that the application of LSD-containing sera to cultures of oligodendroglia and other cellular supporting elements caused a state of contraction lasting for several minutes, followed by a relaxation and expansion of these cells which lasted for several hours. The authors found that if the LSD-containing sera were removed and replaced by the normal media, that the above effects were usually reversed.

The above findings suggest an attractive and obvious explanation as to mechanism of action of LSD. These observable structural changes may well reflect functional alterations at the sub-cellular level, and thus explain altered behavior. As the oligodendroglia have been implicated in the so-called blood-brain barrier, as well as in the cerebral micro-circulation, any alteration in their functional integrity could affect neuronal activity (and thus behavior, state of consciousness, etc.) in one or both of the following ways:

1. A breakdown in the blood-brain barrier could allow chemical neurotoxins from the general body circulation to enter the cerebral microcirculation. Melander (35) has noted that his work suggested that LSD has the property of enabling certain intravenously administered drugs to act on selected brain centers not normally accessible to them - thus endogenous compounds normally present in the blood might also produce behavioral changes through the same mechanism.

2. A change in the pumping mechanism of the oligodendroglia could cause a slowdown in the delivery of essential metabolites to the neurons, or could result in stasis and accumulation of toxic end products of metabolism.

To date, it has been technically impossible to substantiate these in vitro findings by similar observations in the intact experimental animal; consequently, conclusions drawn from these studies must be guarded.

It is quite possible that the structural changes noted in cell culture studies do not adequately or accurately reflect the action of LSD in the intact animal. Thus it is conceivable that LSD exerts its effect on the subcellular level by interfering with essential biochemical processes in the neuron itself or in its supporting elements. Sankar et al. (40) found that LSD interferes with normal phosphorus metabolism, resulting in increased levels of phosphorus in the blood, as well as a decrease in its urinary excretion. He felt that these effects of LSD may be significant and related to its psychosomatic action

because of the similar findings noted in childhood schizophrenia. The manner in which LSD affects cerebral carbohydrate metabolism has been investigated, but the results have been inconclusive and contradictory. Most workers (7) have found that LSD inhibits carbohydrate metabolism, both *in vivo* and *in vitro*; however, Sankar *et al.* (40) found that LSD enhanced the oxidation of glucose in cerebral homogenates.

Interference With Neurohumors

A third possible mechanism of action is that LSD may interfere with neurohumors - *i.e.* - acetyl choline, serotonin, norepinephrine, histamine or others. LSD could disrupt the normal metabolism of brain amines at one or more points in their pathways - *i.e.* - synthesis, transport, binding, site of action, degradation, etc.

In 1952, Poloni and Mafferzzoni (37) found that LSD caused a significant increase in brain acetyl choline levels (although Pepeu, working with Freedman and Giarman (25) was unable to confirm this finding), and it was postulated that some of the psychic effects of LSD might be due to an interference with cholinesterase enzyme systems. In 1955, the *in vitro* studies of Thompson *et al.* (46) demonstrated that LSD was a relatively powerful inhibitor of non-specific or pseudo-cholinesterase in human serum and brain homogenates, but that the true cholinesterase of brain tissue was only slightly affected by concentrations of LSD that almost completely inhibited the non-specific form

of the enzyme. Goldberger (26), however, in 1962, used histochemical techniques and found that LSD did not inhibit pseudocholinesterase and that specific cholinesterase was inhibited only in the cerebral cortex, most markedly in the lamina ganglionaris.

In 1954, Wooley and Shaw (47) published their classic work which implicated serotonin as the agent responsible for the mental disturbances initiated by LSD, basing their hypothesis on the observation that LSD interfered with the action of serotonin (5-hydroxytryptamine, 5-HT) on various smooth muscles of the body. Their hypothesis was that "The suppression of its (i.e. - 5-HT) action results in mental disorder - in other words, it is the lack of serotonin which is the cause of the disorder." The serotonin hypothesis has been vigorously investigated since this initial observation more than a decade ago. It was shown that BOL (D-2 brom - LSD), a monobrom derivative of LSD, was a more powerful peripheral antagonist of serotonin than LSD was, while it lacked the psychotomimetic effect of LSD - thus apparently disproving the original hypothesis of Wooley and Shaw. However, Freedman and Giarman (24) found a small but statistically significant increase in cerebral serotonin levels in experimental animals, indicating that interference in serotonin metabolism may be important in the mechanism of action of LSD. Sankar et al. (41) have reported an overall increase in the metabolism of serotonin in all parts of the body examined, except the cerebrum, subsequent to the administration of LSD.

Experimental Methods

I. LSD Concentration in Whole Guinea Pig Brain

In the determination of the LSD concentration in whole brain (and in the other phases of this study), adult male guinea pigs weighing 400 to 950 grams were used. After weighing each animal on an Ohaus triple-beam balance, the calculated amount of LSD was injected intraperitoneally with a glass syringe fitted with a 25 guage, 5/8 inch sterile disposable needle, and, at the appropriate time, the animal was sacrificed by decapitation; the head was then placed in ice until the remainder of the animals were sacrificed. The brains were then removed from the cranial vaults, and as much as possible of the meninges and superficial blood vessels were dissected away. Each brain, minus the cerebellum, brachia, and proximal spinal cord, was weighed on a torsion balance and again placed in ice, pending analysis for LSD content according to the method of Axelrod (7).

The first step of the analysis consisted of homogenization, in a glass vessel with a Teflon pestle whose original tolerance was 0.0003 in. Each brain was homogenized in (15-brain weight in grams) ml. of 0.3M sucrose (prepared by placing 102.6 grams of sucrose, formula weight 342, in a one-liter volumetric flask and adding distilled water). The homogenization procedure itself involved ten strokes of the homogenizer.

A 5.0 ml. aliquot of this homogenate was then transferred to a Teflon-capped glass centrifuge tube containing the following analytical grade reagents:

a. 24.5 ml. of washed n-heptane

b. 0.5 ml. of iso-amyl alcohol

c. 3.0 gm. NaCl

d. 0.5 ml. 1 N NaOH

The tubes were shaken for thirty minutes and then centrifuged at 2000 RPM for 5 minutes in an International Size II centrifuge. Upon completion of centrifugation, 20 ml. of the n-heptane phase was transferred to a second centrifuge tube containing 3.5 ml. of 0.004 N HCl and was shaken for 15 minutes and centrifuged for 10 minutes at 2200 RPM. The n-heptane phase was then aspirated and 1.0 ml. of the LSD-containing acid layer was transferred to a quartz cuvette. LSD content was determined by measuring its fluorescence in an Aminco spectrofluorophotometer at an activating wave length of 325 millimicrons and a fluorescence wave length of 445 millimicrons.

With Axelrod's method (7), it is necessary to prepare and determine fluorometric readings for a "blank" and a graduated series of "standards." The blank is prepared by adding 5.0 ml. of 0.3 M sucrose without LSD to a centrifuge tube containing all of the reagents in the first step of this procedure (n-heptane, etc., as per above). The "blank" tube is then carried through the subsequent steps along with the tubes containing the brain samples and is finally read in the Aminco fluorometer, and the "blank" value is obtained. The standards are prepared by adding carefully measured increasing amounts of LSD to the quantity of 0.3 M sucrose necessary to give

a 5.0 ml. aliquot, and adding these aliquots to different tubes containing the reagents used in the first step of the analysis (n-heptane, etc.). The LSD used in this study was prepared by the Sandoz Pharmaceutical Company, and was supplied in vials containing 1 milliliter of a 100 microgram per milliliter solution of LSD. For the preparation of the necessary standards, this relatively concentrated solution was diluted to give one containing 10 micrograms per milliliter, which was stored in the frozen state between experiments. At the time of the actual preparation of standards, this solution was further diluted to either 1 microgram or 100 nanograms per milliliter, and different aliquots of these final solutions were added to the standard tubes as described above. These "standard" tubes are carried through the subsequent steps of the procedure, along with the brain samples and the blank, and are ultimately read in the fluorometer.

The fluorometric readings are converted into absolute values of LSD content in the following manner. First a standard curve or graph is constructed, with the amount of LSD as the abscissa and the fluorometric reading as the ordinate - the "blank" value (i.e., zero LSD content) and the graduated LSD "standards" are used to construct the actual standard curve of this graph. The LSD content of the brain samples can then be determined from this graph by finding the point of the standard curve which corresponds to the fluorometric reading of the sample and then directly reading the LSD content

on the abscissa of the graph. This value represents total LSD content of the brain sample; the concentration, or amount of LSD per gram of brain tissue, is determined by dividing the total LSD content by the weight of the brain sample.

II. LSD Concentration in Selected Areas of the Guinea Pig Brain

In the second set of experiments, the animals were injected, sacrificed, and the brains removed, in the manner described above. The regions chosen for analysis were:

a. caudal brain stem, including the medulla, pons, and mesencephalon

b. diencephalon

c. hippocampus

The samples were weighed and homogenized in (5-sample weight) ml. of 0.3 M sucrose. The entire 5 ml. homogenate was then analyzed for LSD content according to the method of Axelrod.

III. LSD Concentration in Guinea Pig Plasma

To determine the LSD concentration in plasma, the animals were injected intraperitoneally with LSD and decapitated at the appropriate time. Blood, mainly from the carotid arteries, was collected directly from the animal into a beaker containing five drops of a saturated solution of EDTA (ethylene diamine tetraacetic acid). The anti-coagulated blood was then centrifuged at 1200 RPM for 10 minutes. One milliliter of the plasma was analyzed for LSD content according to the method described above.

Experimental Results

The purpose of these experiments was to measure the uptake and distribution of LSD in the brain of the guinea pig.

I. LSD Concentration in Whole Guinea Pig Brain

In studying whole brain concentrations of LSD, both the time from injection to sacrifice and the per-kilogram dosage of LSD were used as variables and the results were plotted with time (since injection) on the abscissa and brain concentration of LSD (in nanograms per kilogram) on the ordinate.

The per-kilo doses of LSD employed were quite high when compared to the quantity ordinarily administered to human subjects - *i.e.*, 1-2 micrograms per kilogram, but were felt to be ideal for the guinea pigs used in this study because:

A. These doses resulted in observable behavior changes in the animals.

B. Smaller amounts would have resulted in brain levels too low to be accurately measured over an extended period of time by the technique employed. The doses used were 100, 250 and 500 micrograms of LSD per kilogram of guinea pig weight.

The time from injection to sacrifice was the second variable; the times chosen for use were 5, 10, 15, 20, 40 and 90 minutes. These times were used because:

A. It had been observed early in the course of this study that the most obvious behavioral changes in the guinea pig occurred during the first twenty minutes following injection and then became much less detectable as the animal rapidly resumed what appeared to be normal behavior. Thus it was deemed desirable and necessary to have multiple readings during this critical early period.

B. It has been reported in the literature that whole brain concentrations of LSD in various species - i.e., dog, rat, etc. rapidly reached a peak level and then fell so that very little was detectable at one hour from injection. Therefore, several late readings (40, 60 and 90 minutes) were made to see if the pattern in the guinea pig corresponded to the observations made in other species.

Three curves (see Graph No. 1) were constructed, one for each of the per-kilo doses used. At each point in time at least four animals were studied (average (N) is 8). A total of 143 animals were used in this phase of the study. The results indicate:

A. The whole brain concentration is directly related to the amount of LSD administered (Tables No. 1 and No. 2). It was observed that at all points of time studied during the first forty minutes following injection, the levels are significantly different for the three dose schedules used. After forty minutes, as the levels of LSD decreased further, the differences remained, but became less significant.

B. At each per kilogram dose, the LSD concentration in brain varies with time (Table No. 1). The concentration rises rapidly during the first ten minutes following injection, remains at essentially a plateau level for the next ten minutes, and then begins to decline at a rate considerably slower than its rise. The reason for this relatively slower rate of clearance is not known.

As regards the "plateau" period (Graph No. 1) at 10-20 minutes after injection, it is interesting to note that with the lowest dose employed (100 milligrams per kilogram), the values obtained at 10, 15 and 20 minutes were very similar and a true plateau was observed. At 250 milligrams per kilogram, a slight, statistically insignificant fall occurred at mid-plateau (15 minutes after injection). However, with the highest dose used (500 milligrams per kilogram), a ten percent fall occurred at mid-plateau (15 minutes after injection), $P<0.2$, with a subsequent smaller rise in LSD concentration at 20 minutes ($P<0.6$). Coquet and Freedman observed a similar pattern in the rabbit, but not in the rat. The reason for this fluctuation at the period of peak concentration is uncertain.

C. During the period of 20-40 minutes after injection, there is essentially a fifty percent decrease in concentration at all three dose levels (more precisely, sixty, forty-nine, and fifty-two percent, respectively).

D. Of the total amount of LSD administered, approximately 0.0006 of this quantity was present in the brain at 10 minutes following injection,

[~~concentration.~~] Since the brain constituted an average of 0.006 of the total body weight, the brain concentration, at each dosage level, represented approximately ten percent of the total body concentration, if it were assumed that all of the injected LSD were absorbed from the peritoneal space and distributed evenly in the body mass.

E. The peak concentration of LSD in the brain occurs at the time when behavioral changes in the animal are most obvious. These changes include grossly visible muscle twitching, staring, excitation, incoordination, and apparent confusion.

II. Regional Concentration of LSD

The second phase of this study involved the determination of LSD concentrations in selected areas of the guinea pig brain; the regions chosen for investigation were the caudal brain stem, the diencephalon, and the hippocampus. Animals were injected with the three per kilogram doses used in Part I - i.e., 100, 250, and 500 micrograms of LSD per kilogram, and determinations of regional concentrations were performed at 5, 20, and 40 minutes after injection. The following results were noted:

A. Relation of LSD Concentration to Time (Dose and Region Constant) - Graph No. 2, Table No. 4.

With the two higher doses of LSD, the concentration of LSD in each region was highest at 20 minutes, while the values at 5 and 40 minutes were

significantly lower and nearly identical. This pattern was not seen with the lowest dose, where readings in the diencephalon and caudal brain stem were nearly identical at the three times investigated. The hippocampus, however, reached its peak value at five minutes and then fell 33% by 20 minutes ($P<0.3$), and a further 27% by 40 minutes, for an overall decrease of 60% ($P<0.03$).

B. Relation of LSD Concentration to Brain Region (Dose and Time Constant) - Graph No. 2, Table No. 3.

For each dose, the levels in the regions studied were essentially equal at five minutes and 40 minutes. An apparent exception to this occurred with the lowest dose in the hippocampus at five minutes - here the value for the hippocampus was 29 nanograms per gram, while the value in the other two regions was 16 nanograms per gram. Although the hippocampal value was nearly double the other levels, the significance of this difference (P) was only 0.2.

At 20 minutes, with the two higher doses, the mean values for the three areas showed some regional variation, but only at the 250 microgram per kilogram dose did the difference between two areas (caudal brain stem and hippocampus) reach a significance of 0.01.

C. Relation of Concentration to Amount of LSD Administered - Graph No. 2, Table No. 3.

The concentration of LSD in all areas was seen to be significantly related to the dose of LSD administered except at five and at forty minutes

between the two lower doses. The peak values in the regions studied were approximately four times higher than the total brain concentration at the highest dose; three times higher at the 250 microgram dose and only twice as high at the 100 microgram dose.

III. Plasma Levels of LSD

Animals were injected with either 250 or 500 micrograms of LSD per kilogram of weight and sacrificed at ten, forty or ninety minutes after injection, at which time the plasma was analyzed for LSD content. Approximately three animals were used for each point on the two curves constructed. Results indicated that:

A. At each dose, the concentration of LSD in the plasma varied with time - Graph No. 3, Table No. 5.

B. Plasma levels of LSD varied with the quantity of LSD administered, but not as significantly as had been anticipated (Table No. 5, Graph No. 3). The values at forty minutes were nearly identical, while at ten minutes, the value at 500 micrograms per kilogram was only one-third greater than the smaller dose, instead of the predicted, or anticipated, one hundred percent difference; the difference at ninety minutes was only fifty percent.

C. The peak values for the plasma levels of LSD were approximately eight times the peak whole brain concentrations at 500 micrograms per kilogram, and eleven times the level at the lesser dose.

IV. Additional Observations

When the experimental results from the different phases of this study are analyzed collectively, several additional interesting observations can be made:

A. Relation of Plasma Levels to Whole Brain Levels of LSD (Table No. 6).

At 10 minutes, when the whole brain concentration of LSD is entering the plateau period of peak values, the plasma-brain ratio of LSD is 11.2:1 with the 500 µg/kg, and 7.7:1 with 250 µg/kg dose. At 40 and 90 minutes, when the levels of LSD are falling in both the plasma and the whole brain, the ratio is 5.9:1 and 6.1:1 with the higher dose, and 3.7:1 and 4.1:1 with the lower dose. These results may indicate that the concentration of LSD in the brain is at least partly dependent upon a high concentration gradient between the brain and the plasma and that when this gradient is reduced below a critical value (possibly 7:1) the whole brain levels begin to decline. It may also indicate that the transfer of LSD across the so-called blood-brain barrier is not a purely passive phenomenon.

B. Whereas other investigations have reported that brain levels of LSD were negligible in other species at one hour, considerable levels of LSD were found in the guinea pig brain at 90 minutes - these levels ranged between 20 and 25% of peak values (10 minutes) for the three doses used.

C. In the whole brain, the values at 5 minutes and 40 minutes were nearly identical at all 3 doses. It was interesting to note that this was also true of the various regions of the brain, indicating that LSD is metabolized and cleared from different areas of the brain at the same rate.

D. As Rothlin (39) observed in the cat, the level of LSD in the plasma was at all times measured considerably higher than the whole brain concentration of LSD (and also higher than any regional level of LSD measured).

Discussion

These experiments were undertaken to measure the uptake and distribution of LSD in the brain of the guinea pig at various per kilogram dosages over an extended period of time. The results supplied answers to several immediate questions and may serve as a basis for further allied investigation.

The determination of LSD levels in the whole guinea pig brain was performed to determine the significance of the variables time and dose on brain levels of LSD and to correlate these levels with observable behavioral changes in the animals used. Results clearly demonstrated the effect of these variables on brain levels of LSD and on behavioral changes in the animals.

As a result of this phase of the study, several interesting questions arose, which may merit further investigation to determine:

a) the significance of the plateau found at the time of peak LSD levels in the brain and the reasons for the fluctuation of LSD values seen during this plateau period with the higher doses;

b) the reason for the relatively slow clearance of LSD from the brain, as compared to its rapid initial rise.

The data accumulated should also be of value in future work designed to correlate LSD levels with changes in brain amine metabolism, as well as in the investigation of additional regions of the brain.

The various regions of the brain were studied to determine if LSD were selectively concentrated in different brain areas, thus possibly indicating an important site of action for the drug. Results indicated that LSD was not significantly concentrated by any of the regions studied; however, it is possible that newer techniques in microassay, such as the use of C^{14} labelled LSD, or more careful and finer dissection, may reveal selective localization in the areas examined in this study that was not detectable by the techniques employed. Furthermore, more areas of the guinea pig brain must be examined before the hypothesis of regional localization can be discarded.

The study of plasma levels of LSD indicated that neither the whole brain nor any of the regions studied was able to achieve levels of LSD equal to those in the plasma at any given time - i.e., as Rothlin (39) noted in the cat, plasma levels were always considerably higher than those in the brain; this may indicate that the passage of LSD from plasma to brain is mainly on the basis of passive diffusion. It was found that the plasma:brain ratios of LSD were high at 10 minutes, but were lower and remained constant at 40 and 90 minutes, indicating that an equilibrium had been established; this equilibrium may mean that clearance of LSD from the brain is dependent on clearance of LSD from the plasma which occurs mainly in the liver.

Freedman et al. (19) have shown that following reserpine pretreatment of rats there is less LSD in the brain but an enhanced effect of LSD on behavior. Accordingly, the precise meaning of the presence of the drug - i.e., the

cerebral quantity of LSD at a receptor required for an effect - has yet to be determined. This phenomenon may be explained on the basis of altered brain amine metabolism and must be investigated before a complete understanding of the significance of LSD brain levels can be anticipated.

Graph I

WHOLE BRAIN LEVELS OF LSD

WHOLE BRAIN LEVELS OF LSD: DOSE AND TIME CURVES

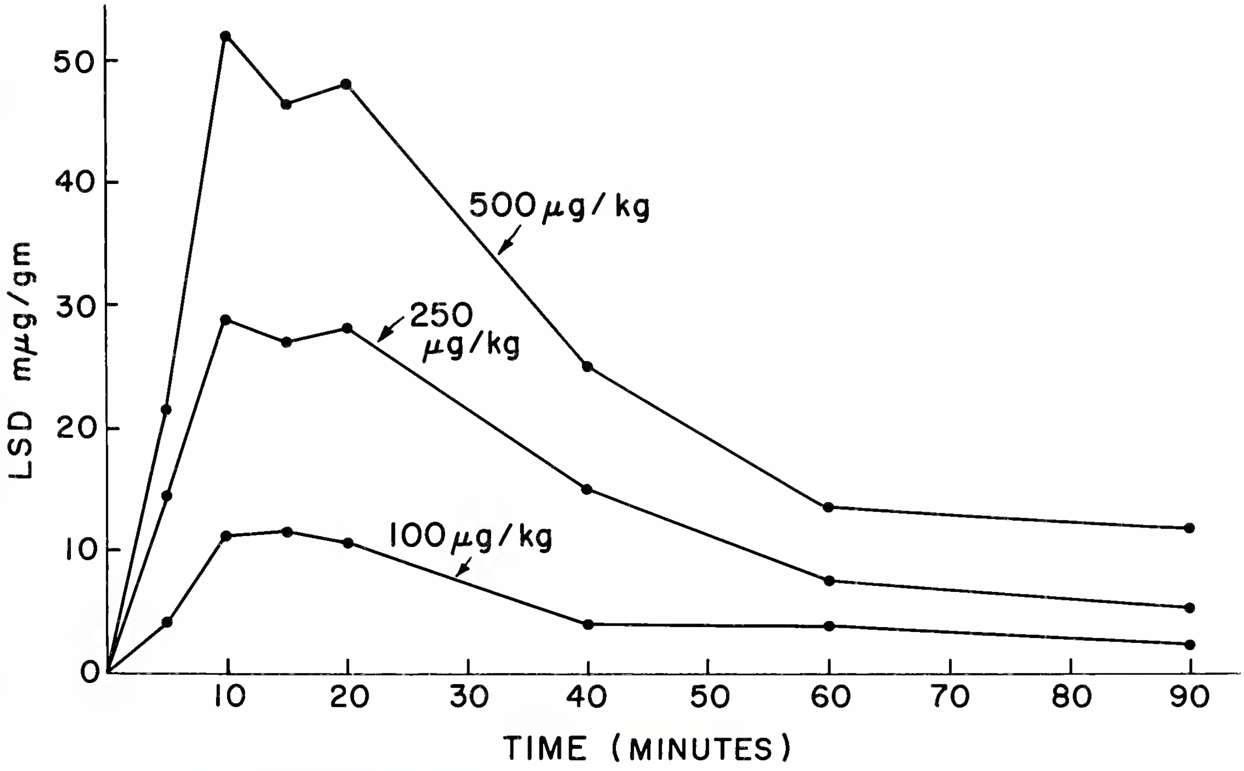

Table I

CHANGES IN WHOLE BRAIN LEVELS OF LSD WITH TIME

	100 ug LSD/kg				250 ug LSD/kg				500 μg LSD/kg			
Time in Minutes	N	$\bar{X}$	S.D.	P	N	$\bar{X}$	S.D.	P	N	$\bar{X}$	S.D.	P
5	4	4.3	.67		4	14.7	4.6		4	21.5	7.1	
				<.01				<.001				<.001
10	8	11.4	4.6		5	28.8	2.6		5	52	7.6	
				<.9				<.4				<.2
15	8	11.6	3.0		5	27.0	3.6		9	46.3	5.3	
				<.6				<.7				<.6
20	10	10.7	3.3		11	28.2	8.5		19	48	9.8	
				<.001				<.01				<.001
40	9	3.8	2.2		7	15	6.5		3	25	3.5	
				<.9				<.02				<.01
60	4	3.9	.95		6	7.6	.55		6	13.5	3.9	
				<.1				<.05				<.6
90	4	3.6	.58		6	5.6	2.0		6	12	3.9	

N = Number of brains used

$\bar{X}$ = Mean concentration in nanograms per gram

Table II

CHANGES IN WHOLE BRAIN LEVELS OF LSD WITH VARYING DOSE

Time in Minutes	100 µg/kg	P	250 µg/kg	P	500 µg/kg
5		<.01		<.2	
10		<.001		<.001	
15		<.001		<.001	
20		<.001		<.001	
40		<.001		<.02	
60		<.001		<.01	
90		<.01		<.01	

Graph II

REGIONAL BRAIN LEVELS OF LSD

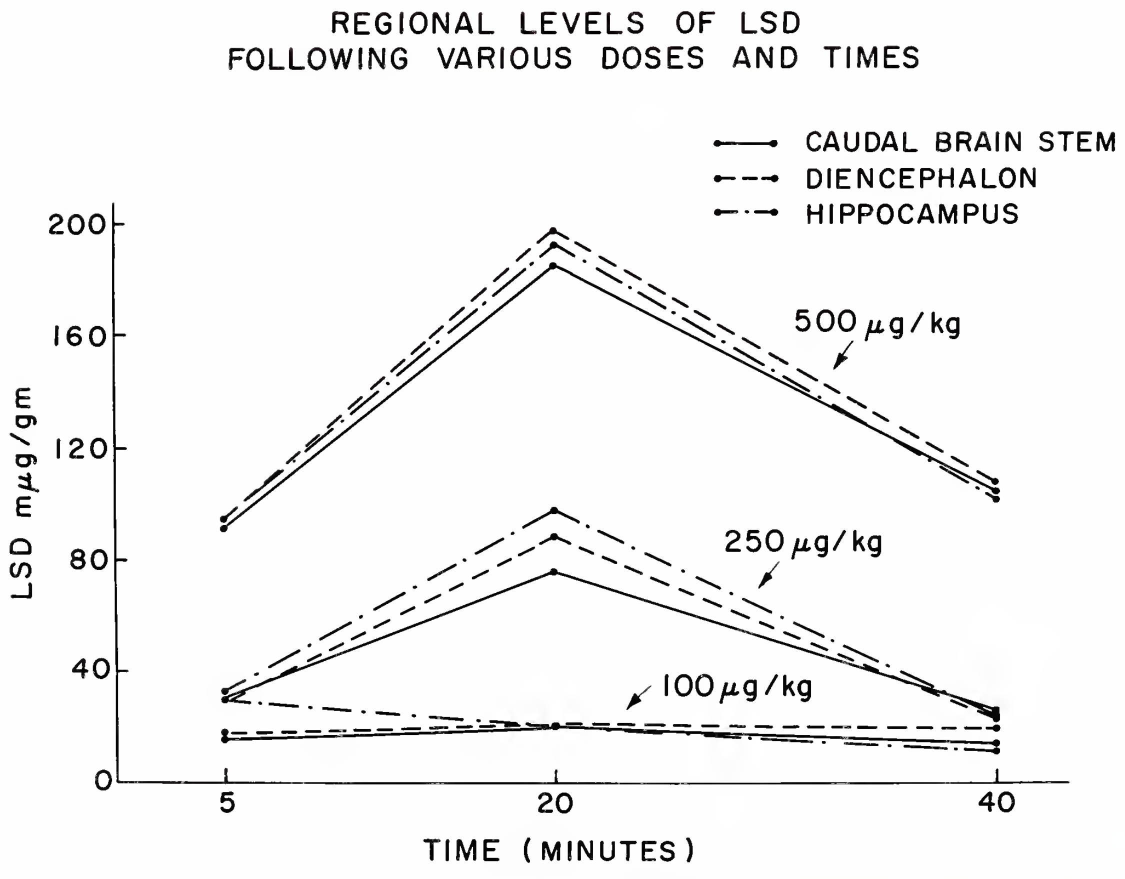

Table III

DIFFERENCES IN REGIONAL CONCENTRATION OF LSD BY VARYING DOSAGE

Time in Minutes	CAUDAL BRAIN STEM					DIENCEPHALON					HIPPOCAMPUS				
	Dose	N	$\overline{X}$	S.D.	P	Dose	N	$\overline{X}$	S.D.	P	Dose	N	$\overline{X}$	S.D.	P
5	100	4	15.7	6.2		100	5	17.6	4.4		100	5	30	16	
					<.1					<.2					<.8
	250	4	31	13.3		250	7	28	19.1		250	7	33	16	
					<.001					<.001					<.001
	500	6	91	15.6		500	5	94	25.1		500	4	94	14	
20	100	7	19.8	8.0		100	6	25	14		100	5	20	11	
					<.001					<.001					<.001
	250	8	76	12		250	8	89	17		250	7	98	14	
					<.001					<.001					<.001
	500	7	186	21.6		500	6	198	20		500	6	193	25	
400	100	6	13.5	3.8		100	6	20	8.3		100	6	11	2.3	
					<.01					<.6					<.1
	250	6	26	6.2		250	5	23	7.0		250	4	25	14	
					<.001					<.001					<.001
	500	5	104	26		500	5	106	20		500	5	102	13	

N = Number of samples
$\overline{X}$ = Mean concentration of LSD in nanograms per gram

Table IV

DIFFERENCES IN REGIONAL CONCENTRATION OF LSD BY VARYING TIME

	100 μg/kg					250 μg/kg					500 μg/kg				
Region	Time	N	$\overline{X}$	S.D.	P	Time	N	$\overline{X}$	S.D.	P	Time	N	$\overline{X}$	S.D.	P
CBS	5	4	16	6.2		5	4	31	13		5	6	91	16	
					<.4					<.001					<.001
"	20	7	20	8.0		20	8	76	12		20	7	186	22	
					<.1					<.001					<.001
"	40	6	14	3.8		40	6	26	6		40	5	104	26	
D	5	5	18	4.4		5	7	28	19		5	5	94	25	
					<.3					<.001					<.001
"	20	6	25	14		20	8	89	17		20	6	198	20	
					<.5					<.001					<.001
"	40	6	20	8.3		40	5	23	7		40	5	106	20	
H	5	5	30	16		5	7	33	16		5	4	94	14	
					<.3					<.001					<.001
"	20	5	20	11		20	7	98	14		20	6	193	25	
					<.2					<.001					<.001
"	40	6	11	2.4		40	4	25	14		40	5	102	13	

N = Number of samples

$\overline{X}$ = Mean concentration of LSD in Nanograms per gram

Graph III

PLASMA LEVELS OF LSD

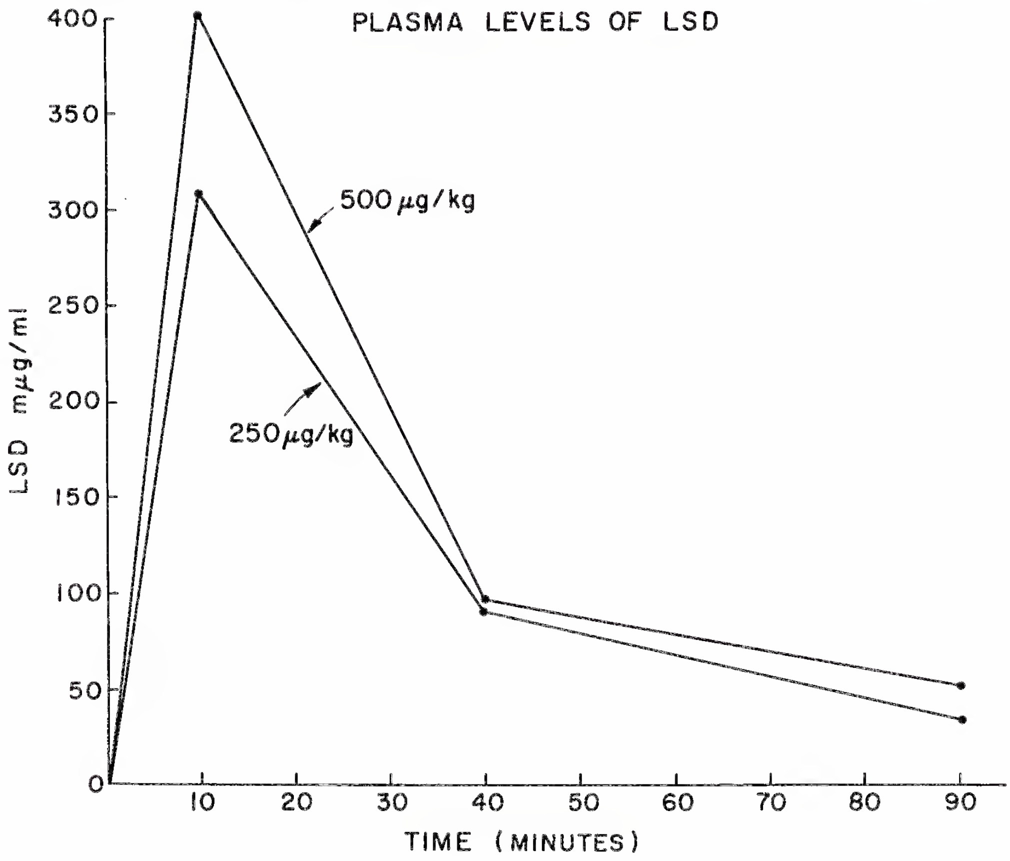

Table V

PLASMA LEVELS OF LSD

I. Relation to Dose

Dose	Time	N	$\bar{X}$	S.D.	P
250	10	3	322	46	<.1
500	10	2	403	10.5	
250	40	3	89	9.3	<.8
500	40	3	93	15.6	
250	90	4	34	10.9	<.1
500	90	3	55	14.1	

II. Relation to Time

Time	Dose	N	$\bar{X}$	S.D.	P
10	250	3	322	46	<.001
40	250	3	89	9.3	<.05
90	250	4	34	10.9	
10	500	2	403	10.5	<.01
40	500	3	93	15.6	<.01
90	500	3	55	14.1	

N = Number of samples

$\bar{X}$ = Mean concentration of LSD in nanograms per ml. of plasma

Table VI

RELATION OF PLASMA LEVELS OF LSD TO WHOLE BRAIN LEVELS OF LSD

Time	Dose/kg	Brain Level (nanograms/gram)	Plasma Level (nanograms/ml)	Ratio	R/R
10	250	28.8	322	11.2	1.45
10	500	52	403	7.7	
40	250	15	89	5.9	1.59
40	500	25	93	3.7	
90	250	5.6	34	6.1	1.48
90	500	12	55	4.1	

Ratio = Plasma level/brain level
R/R = The ratio of the two ratios at a given time

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